# THE NUTRITIONAL REQUIREMENTS OF CERTAIN INSECTS INFESTING CEREALS AND THEIR PRODUCTS

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BY

MARION DEYOE SWEETMAN, B.S., M.S.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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MARION DEYOE SWEETMAN.

# THE NUTRITIONAL REQUIREMENTS OF CERTAIN INSECTS INFESTING CEREALS AND THEIR PRODUCTS.*

By MARION DEYOE SWEETMAN.

Our increasing understanding of the complexity of food constituents and the nutritional requirements of higher animals might be considerably accelerated if the science had a more complete evolutionary foundation. If, as Bayliss (1) maintains, increasing complexity of organisms in the course of evolution is associated with lessened ability to synthesize the compounds of which they consist, lower animals should be expected to have the simpler and hence more easily understood food needs. Actual knowledge does not permit application of this generalization to the succession of forms in the animal kingdom at this time but the recent work of Wulzen (2) on the nutrition of planarian worms is, no doubt, a forerunner of future ventures into this field.

It is the purpose to present in this paper certain results from a more extended study of the nutritional requirements of the confused flour beetle, *Tribolium confusum* Duval, which seem to prove the value of a phylogenetic view-point in the science of nutrition and the usefulness of this species as an experimental animal in biological analysis. The general plan of the experiments has been a study of the effects on growth of modification of food materials by extraction and of rations consisting of purified foodstuffs, with several series and to apply this knowledge in quantitative and qualitative analyses of growth-promoting content.

## REVIEW OF LITERATURE.

A few investigators have attempted to determine the nutritional requirements of certain insects, desiring to understand their environments

* A paper based on this thesis has appeared in the *J. Biol. Chem.*, 1928, lxxvii, 33.

more completely when they were used in other types of experiments, genetic and economic control especially, and in some cases appreciating possible value as subjects in general nutritional studies. The small amount of food required, short life cycles, and simple laboratory requirements for rearing have been attractive characteristics.

Of insects thus studied, the fruit fly, *Drosophila melanogaster*, has received most attention. This species normally lives upon decaying fruit but Delcourt and Guyenot (3) have reported a method of securing aseptic colonies reared upon potato and dead bakers' yeast while the potato alone produced few, stunted, and sterile adults. Guyenot (4) further found that they could reproduce upon yeast alone and a synthetic diet containing yeast or its alcoholic extract and suggested the similarity of the essential fraction of yeast to Funk's "vitamine." Loeb and Northrop (5) and Northrop (6) verified these findings regarding the value of yeast and its extract in diets for this insect. Baumberger (7) in an extensive study thought the essential portion of yeast was its nucleoprotein but the growth-promoting substance may have been present in his preparation. Glaser (8) thinks that fresh, uncontaminated, orange or grapefruit juice contains an essential substance destroyed by his method of sterilization. Most recently Bacot and Harden (9) using a purified basal diet consisting of caseinogen, starch, salts, and cane sugar, all extracted with alcohol, reared aseptic cultures of the flies when yeast or the alcoholic extract of wheat germ was added with butter fat and with or without fresh lemon juice. They were not certain whether the butter fat was necessary or not.

Other insects living upon decaying material whose nutritional requirements have been investigated are the blow flies studied by Bogdanow (10) and Wollman (11) with experiments inadequately planned for conclusive results.

Another type of insect, that infesting cereals and their products, from the nature of its food habits offers even greater possibilities for such experimental use. *Tenebrio molitor* was studied by Portier (12) who reared its larvæ as rapidly on a sterilized as on an unsterilized medium, thus demonstrating that the presence of microorganisms was unessential, and Passarini (13) who found a favorable influence of the pericarp of cereals. Chapman (14) has conducted extensive research with the confused flour beetle, *Tribolium confusum*, in which he used purified diets consisting of cornstarch, various proteins, salts, and wheat germ or the vitamin B-containing fraction. He concluded that the wheat germ itself was a more satisfactory supplement to his purified basal rations than the vitamin B portion (an alcoholic extract on dextrin) but none of his basal rations contained fat. Evidence will be pointed out in this paper that fat considerably accelerates growth on an otherwise adequate diet. Furthermore, the single purified proteins used were often incomplete, hence the embryo may have helped cover an amino acid deficiency. Such considerations mean that the vitamin B content of the embryo was not demonstrated to be unessential and further evidence to this effect was afforded by the fact that only rarely did an adult emerge on a purified ration lacking the embryo or its extract as sources of this vitamin.

Richardson (15) studied the effect of the extraction of whole wheat with various solvents on the growth of *Ephestia kuehniella*. Since the removal of ether-soluble material retarded or prevented development and the addition of egg yolk extract or the return of the extracted portion permitted it, but olive oil and lard did not, he concluded that a vitamin A deficiency was indicated. It is possible that wheat contains a small amount of this vitamin as Steenbock and Coward (16) have pointed out, but the negative effects reported for butter are not adequately explained. One cannot accept Richardson's negative results in the face of his admission that they were sometimes different when he used purified solvents.[1] He derives a conclusion that vitamin B is necessary because alcoholic extraction hinders development but he reports no extraction drastic enough to prevent all transformations, nor does he say whether the moisture content of these extracted materials was adjusted or not; it is known that this insect is sensitive to this factor.

In general, it may be concluded that the work of Guyenot, Northrop, and Bacot and Harden seems to show that *Drosophila* requires vitamin B but there is no conclusive evidence that vitamin A is essential. The investigations of Chapman and Richardson indicate a need for vitamin B on the part of the two cereal-infesting insects studied but the conclusion cannot be unmistakably derived from the experiments as planned and reasons for doubting the conclusion of the latter, that vitamin A is necessary, have been stated.

## EXPERIMENTAL.

*Tribolium confusum* is a very cosmopolitan member of the coleopterous family Tenebrionidæ. Adults and larvæ are common pests of cereals and allied products. At a constant temperature of 28° and relative humidity of 70 per cent the egg stage lasts about 6 days, the larval period on whole wheat about 21 days, and the pupal period 7 days. These stages can be shortened by raising the temperature and in some of these experiments this was done. The larval period is the period of growth and the adequacy of a ration can be measured by the number of days required from hatching to pupation when other conditions are constant. The full grown larvæ are about $\frac{1}{4}$ inch long.

[1] The writer carried on some experiments with *Ephestia* to parallel those here reported for *Tribolium* but results with the former were not so complete because of decimation of many cultures by disease. However, adult moths have been produced on the wheat embryo extracted with ether as later described in this paper and supplemented with Crisco, a fat lacking vitamin A. Furthermore, the ether-alcohol-extracted embryo of the same series produced a 50 per cent yield of moths in one culture of twenty when supplemented by the alcoholic extract and Crisco.

*Technique of Handling Tribolium.*—A large number of adults are transferred to patent flour that has been sifted with fine bolting cloth (No. 5 XX silk) to remove particles which would otherwise appear with the eggs. The container is kept in a warm place (room temperature or incubator up to 31°) for a day or two and then the beetles can be removed with a coarse sieve. Resifting with the bolting cloth removes the eggs which are readily recognizable as tiny pellets, being covered with a coating of flour which adheres to them upon laying. These are placed in an incubator and upon the day of hatching are transferred by means of a camel's-hair brush to the vials containing the experimental rations. 24 × 90 mm. homeopathic vials containing approximately 5 gm. of food are adequate for at least twenty larvæ. These containers are plugged with absorbent cotton and retained in an incubator until pupation occurs. Duplicate vials are prepared for each test.

In the present experiments we used in some cases a Carrier cabinet maintaining a relative humidity of 70 per cent by the spray process; in others a constant temperature incubator in which the bottom was covered with a pan of saturated NaCl solution which maintains a relative humidity of about 75 per cent was found to be satisfactory. This difference in relative humidity did not seem to be significant. Constant temperature facilitates comparison of results run at different times or differing in the length of time required until pupation. As the time for pupation approached the cultures were examined daily by emptying the contents of the vial onto a sheet of paper. Transformations could thus be easily observed, recorded, and pupæ removed. The remaining larvæ were poured with the food back into the vial.

The moisture content of all of the rations was made up to approximately 10 per cent but this is unnecessary if the foodstuffs are held at the above relative humidity for 2 or 3 weeks before larvæ are added since an equilibrium takes place which gives an adequate moisture content.

*Analysis of Nutritional Requirements of Tribolium.*

*Experiment 1. Effect of Ether and Alcohol Extraction of Wheat Embryo upon Growth.*—Commercial wheat embryo was found to be an optimum food for this insect and hence was made the basis of a series of experiments to determine as definitely as possible the essential constituents for growth. The embryo as purchased was

extracted with ether 48 hours, then with cold 90 per cent alcohol, followed by the same hot to a total extraction period of 3 weeks, all in a Lloyd continuous extractor. In making up the experimental rations fat was returned to the amount of 10 per cent, slightly less than the ether extract obtained. The alcoholic extract was evaporated onto dextrin and used as an aliquot of the original. Butter fat was prepared for this work by rendering and filtering to remove the curd and water. Results are given in Table I.

TABLE I.

*Growth of Tribolium on Commercial Wheat Germ and after Extraction with Alcohol and Ether.*

Temperature, 28°; relative humidity, 70 per cent; twenty larvæ per vial.

| Ration No. | Ration content. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| | *per cent* | | | |
| 1 A | Commercial wheat embryo. | 21.2 ± 0.094 | 2.94 | 20 |
| | | 20.8 ± 0.108 | 3.22 | 19 |
| 25 A | Embryo extracted 3 wks. with alcohol and ether. | | | 0† |
| | | | | 0 |
| 26 A | Ration 25 A..... 90 | | | 0† |
| | Butter fat..... 10 | | | 0 |
| 27 A | Ration 25 A..... 90 | | | 0† |
| | Crisco..... 10 | | | 0 |
| 28 A | Ration 25 A + alcoholic extract = 10 per cent embryo. | 30.6 ± 0.384 | 8.10 | 19 |
| | | 28.9 ± 0.305 | 6.59 | 20 |
| 29 A | Ration 28 A..... 90 | 24.1 ± 0.384 | 5.59 | 19 |
| | Butter fat..... 10 | 23.0 ± 0.146 | 4.05 | 20 |
| 30 A | Ration 28 A..... 90 | 26.0 ± 0.298 | 7.45 | 20 |
| | Crisco..... 10 | 25.3 ± 0.238 | 6.22 | 20 |

* Coefficient of variability.

† The same results when repeated.

Since all fat and fat-soluble substances must have been removed from the embryo by this extended extraction it is evident that neither fat nor fat-soluble vitamins are absolutely limiting growth factors. On the other hand, the fact that a return of a 10 per cent equivalent of the alcoholic fraction permits pupation indicates a need for vitamin B. The retardation noted on comparison of this ration (No. 28 A) with the control was almost overcome by the addition of a fat, the presence of vitamin A seeming to be insignificant.

*Experiment 2. Growth on Ration of Purified Constituents with and without Vitamins A and B.*—Since commercial wheat embryo was taken as the control ration for the above experiment a purified foods series was planned with a basal ration approximating the embryo in composition to study the effect of the presence or absence of the two vitamins, A and B. This basal ration contained casein 28, Osborne-Mendel (17) salts 4, fat 10, and dextrin to 100.

TABLE II.

*Growth of Tribolium on Ration of Purified Constituents with and without Vitamins A and B.*

Temperature, 28°; relative humidity, 70 per cent; twenty larvæ per vial.

| Ration No. | Ration content. | | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|---|
| | | | Mean. | C.V.* | |
| | | *per cent* | | | |
| 1 B | Casein | 28 | | | |
| | Osborne-Mendel salts | 4 | | | 0 |
| | Dextrin | 68 | | | 0 |
| 2 B | Casein | 28 | | | |
| | Osborne-Mendel salts | 4 | | | |
| | Dextrin | 58 | | | 0 |
| | Butter fat | 10 | | | 0 |
| 3 B | 2 B with Crisco in place of butter fat. | | | | 0 |
| | | | | | 0 |
| 5 B | Casein | 28 | | | |
| | Osborne-Mendel salts | 4 | | | |
| | Alcoholic extract = germ 10 per cent | | 28.4 ± 0.340 | 7.75 | 19 |
| | Dextrin to | 100 | 29.1 ± 0.340 | 7.56 | 19 |
| 6 B | 5 B with 10 per cent butter fat in place of dextrin | 10 | 26.4 ± 0.408 | 10.00 | 19 |
| | | | 26.8 ± 0.438 | 10.34 | 18 |
| 7 B | 5 B with 10 per cent Crisco in place of dextrin | 10 | 25.5 ± 0.349 | 8.59 | 18 |
| | | | 26.1 ± 0.238 | 5.90 | 19 |

* Coefficient of variability.

Dextrin was prepared from commercial corn-starch by moistening with 0.2 per cent citric acid and autoclaving 5 hours at 15 to 20 pounds pressure. The dried product was ground and extracted with cold 90 per cent alcohol 24 hours, the same hot 48 hours, and ether 36 hours, all in a Lloyd continuous extractor. Casein was prepared as described by Palmer and Kennedy (18).

When yeast is mentioned, reference is to the pure, dried, product

of the Northwestern Yeast Company. A summary of the results on this series of rations is given in Table II.

The similarity (compare Tables I and II) between the pairs, Rations 28 A and 5 B, Rations 29 A and 6 B, and Rations 30 A and 7 B, is so remarkably exact that the same observations apply. Thus it is seen that a purified diet can be prepared for this insect, which gives practically the same growth rate as the equivalent rations prepared from extracted wheat embryo. In the data here presented the growth rate in neither case quite attained that on the untreated embryo.

### *Applications in Vitamin B Studies.*

*Experiment 3. Quantitative Studies of the Effect of Additions of Sources of Vitamin B to a Purified Basal Ration.*—Since these insects seemed to be sensitive to the presence or absence of vitamin B in their food materials an attempt was made to determine whether their growth rate might not be a means of measuring that constituent. The basal ration consisted of casein 28, Osborne-Mendel salts 4, ether extract of wheat germ 3, and dextrin to 100. This percentage of fat was used because of evidence that certain irregular results might be due to a slight excess or failure to incorporate it into the mixture so that there was no greasiness. 3 per cent of fat is approximately the proportion found in whole wheat, another optimum food material. The proportions of sources of vitamin B are plotted against days in the larval period in Fig. 1.

It is evident from the curves that result that this insect responds in a quantitative fashion to amounts of growth-promoting substance over a certain range of proportions. Response was somewhat irregular at the lower levels but this may be partially due to weaknesses in the technique of preparation of the rations. It is probably difficult to secure a uniform distribution of a 1 per cent fraction of such a mixture with a mortar and pestle. All of the ingredients for this series were sifted through fine bolting cloth before mixing and for such refined work no doubt this should have been repeated after combination. Sherman and Spohn (19) say that by feeding their basal diets "to experimental animals of suitable age and size and sufficiently matched as to nutritional history and litter controls, it is believed to be possible, when deal-

ing with averages of ten or more rats on each diet, to detect a diminution certainly of 25 per cent and probably of 15 per cent in the vitamin B content of the food tested." In each case there is a definite decrease in growth rate of the insects when the source of vitamin B is diminished from 2 to 0.5 per cent of the ration and on some curves more sensitivity is indicated by the variation over a

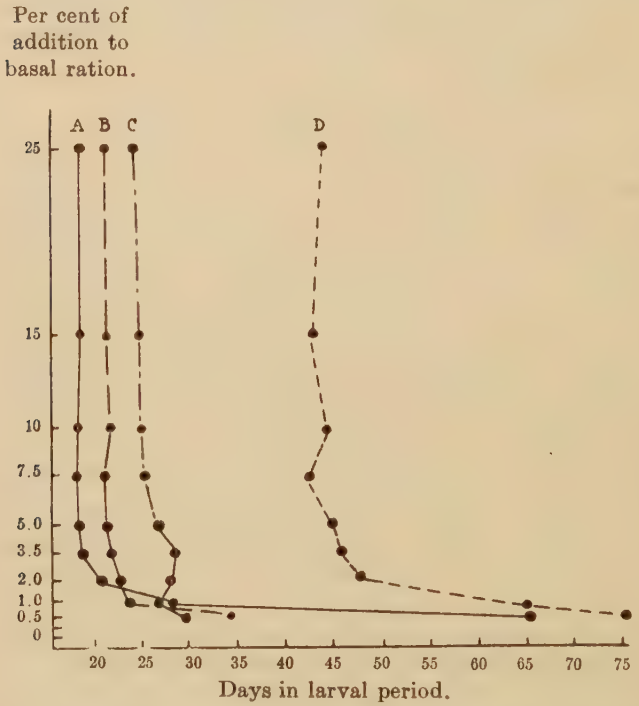


FIG. 1. Growth of *Tribolium* when a source of vitamin B is added to a purified basal ration in varying proportions. Addition to basal ration: *A*, wheat embryo; *B*, alcoholic extract equivalent to wheat embryo; *C*, yeast; *D*, alcoholic extract equivalent to yeast. Basal ration: casein 28, Osborne-Mendel salts 4, ether extract of wheat embryo 3, dextrin 65.

wider range. It is also to be noted that *Tribolium* is remarkably sensitive to very small amounts of vitamin B, as little as 0.5 per cent of a source being sufficient to produce some pupæ.

Another condition lessening exactness is the greater scatter of the individual cases, resulting in a high probable error of the

mean at the low levels, a state found on all rations which are far from optimum. In this connection, it is interesting to find that Sherman and Spohn (19) report for rats that "the coefficient of variation is greatest for the diet containing (of those compared) the smallest amount of vitamin and becomes less as the vitamin intake approaches the optimum." Individual variations were larger on diets permitting only subnormal growth and as these were brought nearer optimum the rate of growth became both more rapid and more uniform.

But an even more outstanding result of this series was the rather unexpected qualitative differences indicated among the four sources of vitamin B which showed no tendency to diminish at high levels of feeding.[2] These differences correlate with the previous failures to secure quite optimum growth on a purified ration supplemented either with yeast or the alcoholic extract of wheat embryo. Wheat embryo seems to contain some growth-promoting substance absent from its alcoholic extract or yeast. This phase of the problem is being investigated further for its relationship to the question of the multiplicity of factors involved in the vitamin B complex. Evidence on this point has been constantly accumulating and has been most recently summarized by Sherman and Axtmayer (20).

*Experiment 4. Use of Tribolium in Following the Chemical Purification of Vitamin B.*—This experiment had a twofold purpose, an attempt to identify more closely the growth-promoting substance found in alcoholic extracts by preparing one of the most purified forms of vitamin B reported in the literature, and an examination of the usefulness of *Tribolium* in following results at each stage in a chemical procedure.

The recent method of Levene and van der Hoeven (21) was followed. This is based upon the discovery that the vitamin B fraction of yeast can be precipitated from water and dilute alcohol solutions by bringing the alcoholic content to about 79 per cent (Osborne and Wakeman (22)) and that further concentration can be secured by adsorption on silica gel at pH 5 and release by increasing acidity to pH 3 or alkalinity to pH 9.5. In these experiments a basal ration was used that consisted of casein 28, Osborne-

[2] The extract of yeast was prepared by Dr. Cornelia Kennedy by extraction in a Soxhlet extractor with 85 per cent alcohol until the extract came through colorless.

Mendel salts 4, Crisco 3, and dextrin 65. To this were added definite amounts of the concentrated material as it occurred at different stages in the process. The residue as well was tested. The cultures were reared in an incubator with little variation from 31° in temperature and 75 per cent relative humidity. These conditions produced pupation in the control wheat germ in 14 to

TABLE III.

*Purification of Vitamin B Fraction of Brewers' Yeast According to Methods of Osborne and Wakeman, and of Levene and van der Hoeven and the Growth-Promoting Power of Product and Residue at Various Stages.*

Temperature, 31–32°; relative humidity, 75 per cent; twenty larvæ per vial.

| Ration No. | Addition to basal ration. | | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|---|
| | | | Mean. | C.V.* | |
| | | *per cent* | | | |
| 14 B | Dried brewers' yeast..... | 10 | 16.3 ± 0.865 | 3.52 | 20 |
| | | | 16.3 ± 0.708 | 2.87 | 20 |
| 15 B | " " " ..... | 1 | 16.9 ± 0.081 | 3.18 | 20 |
| | | | 16.8 ± 0.077 | 2.83 | 20 |
| 16 B | Osborne-Wakeman fraction on dextrin (roughly equal to 30 per cent dried brewers' yeast)... | 10 | 30.1 ± 0.294 | 5.78 | 16 |
| | | | 31.8 ± 0.591 | 12.42 | 16 |
| 17 B | " " | 1 | 30.4 ± 0.421 | 7.41 | 13 |
| | | | 32.2 ± 0.729 | 12.53 | 14 |
| 18 B | Product of the first release at pH 3 dried on dextrin†..... | 10 | 34.5 ± 0.512 | 9.03 | 17 |
| | | | 34.1 ± 0.684 | 11.90 | 16 |
| 19 B | " " | 1 | 32.5 ± 0.729 | 12.85 | 15 |
| | | | 34.6 ± 0.493 | 8.97 | 18 |
| 20 B | Final Levene-van der Hoeven product.‡..... | 2 | 32.0 ± 1.37 | 16.85 | 7 |
| | | | 31.2 ± 1.37 | 14.52 | 5 |
| 21 B | " " | 1 | 32.8 ± 0.762 | 11.05 | 10 |
| | | | 31.2 ± 0.644 | 9.22 | 9 |
| 22 B | " " | 0.1 | 37.0 ± 1.32 | 14.89 | 8 |
| | | | 39.3 ± 1.22 | 14.55 | 10 |
| 23 B | Filtrate after adsorption dried on 10 gm. dextrin (see note to Ration 18 B)..... | 10 | 30.0 ± 0.561 | 10.36 | 14 |
| | | | 30.7 ± 0.923 | 16.10 | 13 |
| 24 B | " " | 1 | 30.8 ± 0.758 | 14.00 | 15 |
| | | | 30.3 ± 0.543 | 11.24 | 18 |

TABLE III—*Concluded.*

| Ration No. | Addition to basal ration. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| | *per cent* | | | |
| 25 B | Final acetone filtrate dried on 10 gm. dextrin (see note to Ration 20 B)..... 10 | | | 2§ |
| | | | | 0 |
| 26 B | " " 1 | | | 5§ |
| | | | | 2 |

* Coefficient of variability.

† The Osborne-Wakeman fraction from approximately 170 gm. of dried brewers' yeast was deaminized by $NO_2$ and the solution subjected to adsorption with silica gel at pH 5. A first release was made at pH 3 and this solution dried on 10 gm. of dextrin. This portion was thus kept separate from later releases to insure greater purity from material carried down mechanically.

‡ The product of a second release when the silica gel adsorption combination was returned to pH 3 after neutralization plus that obtained at pH 9.5 was concentrated and precipitated by dry acetone.

§ Cultures discontinued on 68th day with some larvæ remaining which might have pupated.

15 days, a much accelerated rate over that at 28°. Results are summarized in Table III.

Further evidence is offered by this work that water or alcoholic extracts of yeast are never equal in growth-promoting power to the yeast itself. The time for pupation is doubled and the yield reduced. The presence of the necessary substance in every fraction as this process was developed indicates the source of at least some of the losses reported by Levene and van der Hoeven. It is to be noted that these insects are very well adapted to testing such a chemical purification process because they can detect so noticeably and quickly such small amounts of the growth-promoting material. The fact that the final acetone filtrate was apparently much less abundantly supplied with the vitamin indicates that it is a good precipitant for that factor.

0.1 per cent, 1 part in 1000, of the final product did produce pupæ but the extent of the concentration cannot be judged because the supplementary power of the yeast itself in such a small proportion was not tested. When the experiments were planned it was

expected that the 1 per cent level would be limiting but it was not (see Ration 15 B).

*Experiment 5. Adequacy of Portions of Fractionated Cereals for Growth of Tribolium.*—One phase of the nutrition of this cereal-infesting insect which it was thought might throw some more light upon the requirements of the insect itself as well as furnish data for comparison with what is known of the adequacy of these food materials from research conducted with other animals, is a study of its growth on different cereals and their fractions. All of these fractions were carefully prepared by hand dissection with a sharp dental scalpel and grinding with a mortar and pestle. The part of the experiment which deals with wheat was run at the usual temperature of 28° and 70 per cent relative humidity, that with

TABLE IV.

*Growth of Tribolium on Different Fractions of Wheat Kernel (Triticum vulgare).*

Temperature, 28°; relative humidity, 70 per cent; twenty larvæ per vial.

| Ration No. | Ration content. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| 1 C | Ground whole kernel. | 21.7 ± 0.252 | 7.49 | 20 |
| 2 C | Wheat embryo removed by hand. | 21.2 ± 0.119 | 3.76 | 20 |
| 3 C | Half of kernel near embryo end, embryo removed. | 21.7 ± 0.084 | 2.57 | 20 |
| 4 C | Remainder of kernel. | 25.4 ± 0.188 | 4.79 | 19 |
| 5 C | Patent flour. | 27.3 ± 0.455 | 10.10 | 17 |
| | | 28.3 ± 0.595 | 13.94 | 20 |

* Coefficient of variability.

corn at 30° and 75 per cent relative humidity, and the remainder at 31–32°. Results are given in Tables IV to VIII inclusive.

It is of course evident that this type of experiment does not permit determination of the identity of a limiting factor. All one can say is that some more or less quantitative or qualitative deficiency is indicated by comparison of results but even this might have a certain significance in the case of cereals completely analyzed biologically in the case of mammals. Such applications will be pointed out later.

In the case of wheat some factor in the endosperm is at a growth-limiting level, but whole wheat, the embryo, or the embryo end

of the kernel gives uniformly normal growth (Table IV). In the corn experiment white and yellow varieties were compared with no result indicating a superiority in the yellow due to its vitamin A content. This agrees with previous evidence of absence of

TABLE V.

*Growth of Tribolium on Different Fractions of Corn Kernel (Zea mays).*

Temperature, 30°; relative humidity, 75 per cent; twenty larvæ per vial.

| Ration No. | Ration content. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| 6 C | Whole yellow corn (Minnesota 13). | 29.7 ± 0.691 | 15.45 | 20 |
| 7 C | Whole white corn (Silver King). | 24.6 ± 0.336 | 8.54 | 18 |
| 8 C | Embryo yellow corn. | 27.5 ± 0.746 | 17.52 | 19 |
| 9 C | " white " | 26.1 ± 0.702 | 17.40 | 19 |
| 10 C | Remainder of kernel, embryo removed, yellow. | 29.8 ± 0.417 | 8.94 | 19 |
| 11 C | Remainder of kernel, embryo removed, white. | 26.0 ± 0.270 | 6.58 | 20 |

* Coefficient of variability.

TABLE VI.

*Growth of Tribolium on Different Fractions of Barley (Hordeum vulgare).*

Temperature, 31–32°; relative humidity, 75 per cent; ten larvæ per vial.

| Ration No. | Ration content. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| 12 C | Whole barley kernel (hull removed). | 14.6 ± 0.104 | 3.35 | 10 |
| | | 14.6 ± 0.104 | 3.40 | 10 |
| 13 C | Barley embryo. | 14.5 ± 0.106 | 3.45 | 10 |
| 14 C | Embryo end of kernel, embryo removed. | 14.6 ± 0.093 | 3.03 | 10 |
| | | 14.6 ± 0.093 | 3.03 | 10 |
| 15 C | Remainder of kernel. | 18.7 ± 0.242 | 5.57 | 9 |
| | | 19.0 ± 0.165 | 4.07 | 10 |

* Coefficient of variability.

sensitivity to this factor. Croll and Mendel (23) reported a lack of vitamin B in corn endosperm but removal of the embryo did not consistently prolong the growth of *Tribolium* (Table V). Barley (Table VI) gave normal growth if the embryo or embryo end of the

endosperm was present but the distal end of the kernel produced retarded development when used as the entire ration. This is very similar to the results with wheat. The fractions of the oat kernel (Table VII) were essentially similar in effect but none was

TABLE VII.

*Growth of Tribolium on Different Fractions of Oats (Avena sativa).*

Temperature, 31–32°; relative humidity, 75 per cent; ten larvæ per vial.

| Ration No. | Ration content. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| 16 C | Whole oat kernel (hull removed). | 16.6 ± 0.190 | 3.40 | 10 |
| | | 16.9 ± 0.177 | 4.91 | 10 |
| 17 C | Oat embryo. | 16.4 ± 0.195 | 5.54 | 10 |
| 18 C | Embryo end of kernel, embryo removed. | 16.2 ± 0.159 | 4.61 | 10 |
| | | 15.9 ± 0.212 | 5.59 | 9 |
| 19 C | Remainder of kernel. | 16.8 ± 0.314 | 8.31 | 9 |
| | | 16.8 ± 0.314 | 8 31 | 9 |

* Coefficient of variability.

TABLE VIII.

*Growth of Tribolium on Different Fractions of Rice Kernel (Oryza sativa).*

Temperature, 31–32°; relative humidity, 75 per cent; ten larvæ per vial.

| Ration No. | Ration content. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| 20 C | Whole rice kernel (hull removed). | 17.0 ± 0.135 | 3.71 | 10 |
| | | 17.2 ± 0.085 | 4.32 | 10 |
| 21 C | Polished rice. | 21.8 ± 0.368 | 4.43 | 3 |
| | | 27.0 ± 1.770 | 25.61 | 7 |
| 22 C | Embryo end of polished rice with embryo completely removed. | 31.2 ± 2.47 | 23.45 | 4 |
| | | 40.6 ± 3.22 | 26.24 | 5 |
| 23 C | Remainder of kernel. | 47.0 ± 2.31 | 16.90 | 5 |
| | | 39.7 ± 3.64 | 27.20 | 4 |

* Coefficient of variability.

the equal of whole wheat or barley. Whole rice (Table VIII) is very like oats but it is remarkable that polished rice or the endosperm alone permitted a fair percentage of pupations in spite of the belief that this portion entirely lacks vitamin B (McCollum and Davis (24)). This is probably evidence that these animals

can exist on a lower level of this factor than the usual laboratory animals, possibly because their growth period is more elastic. One is led to speculate on the probability of the presence of this vitamin in all once living structures.

TABLE IX.

*Growth-Promoting Power of Wheat Kernel and Its Fractions When Added to Purified Ration Lacking Vitamin B.*

Temperature, 31–32°; relative humidity, 75 per cent; twenty larvae per vial.

| Ration No. | Addition to basal ration. | | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|---|
| | | | Mean. | C.V.* | |
| | | *per cent* | | | |
| 24 C | Whole wheat | 20 | 17.0 ± 0.201 | 7.42 | 18 |
| | | | 16.4 ± 0.161 | 6.32 | 19 |
| 25 C | " " | 10 | 18.1 ± 0.299 | 10.95 | 20 |
| | | | 19.1 ± 0.322 | 11.23 | 19 |
| 26 C | " " | 1 | 39.2 ± 3.75 | 31.62 | 5 |
| | | | 46.7 ± 3.03 | 27.10 | 8 |
| 27 C | Dissected wheat embryo | 10 | 16.5 ± 0.178 | 6.62 | 17 |
| | | | 16.8 ± 0.185 | 6.59 | 18 |
| 28 C | " " " | 1 | 20.2 ± 0.226 | 7.81 | 20 |
| | | | 20.6 ± 0.293 | 9.44 | 20 |
| 29 C | Embryo end of kernel, embryo removed | 20 | 16.2 ± 0.178 | 7.28 | 20 |
| | | | (Duplicate omitted by mistake.) | | |
| 30 C | " " | 10 | 19.5 ± 0.283 | 9.61 | 20 |
| | | | 17.6 ± 0.138 | 7.84 | 20 |
| 31 C | " " | 1 | 47.4 ± 3.58 | 29.55 | 7 |
| | | | 45.0 ± 2.56 | 21.65 | 6 |
| 32 C | Remainder of kernel | 20 | 23.6 ± 0.395 | 11.10 | 20 |
| | | | 23.4 ± 0.447 | 12.64 | 20 |
| 33 C | " " " | 10 | 30.7 ± 1.145 | 19.10 | 12 |
| | | | (Duplicate omitted by mistake.) | | |
| 34 C | " " " | 1 | 36.0 ± 2.02 | 25.0 | 2 |
| | | | 48.0 ± 2.23 | 11.38 | 3 |

* Coefficient of variability.

*Experiment 6. Use of Tribolium for Determination of Vitamin B in Portions of Cereals Added to a Purified Basal Ration Lacking That Factor.*—Bell and Mendel (25) and Croll and Mendel (23) have made detailed studies of the vitamin B content of wheat and corn

and their fractions using rats and mice as experimental animals. Since previous results with *Tribolium* indicated a sensitivity to this vitamin a group of rations was planned so that results might be comparable. The basal ration and conditions of the experiment were again those of Experiment 4.

The rations and larval periods for the wheat determinations are given in Table IX. The comparative abundance of the vitamin in the embryo is evident from comparative growth on Rations 26 C, 28 C, 31 C, and 34 C. Increase of endosperm distal to the embryo is associated with decrease of vitamin B. This conclusion

TABLE X.

*Growth-Promoting Power of Corn Kernel and Its Fractions Added to Purified Basal Ration Lacking Vitamin B (Yellow Corn).*

Temperature, 31–32°; relative humidity, 75 per cent; twenty larvæ per vial.

| Ration No. | Addition to basal ration. | Days in larval period. | | No. of pupæ. |
|---|---|---|---|---|
| | | Mean. | C.V.* | |
| | *per cent* | | | |
| 35 C | Whole corn..... 20 | 21.4 ± 0.218 | 6.45 | 19 |
| 36 C | " " ..... 10 | 21.5 ± 0.188 | 5.02 | 15 |
| 37 C | " " ..... 1 | 34.7 ± 1.38 | 17.75 | 9 |
| 38 C | Corn embryo..... 20 | 21.0 ± 0.289 | 8.92 | 19 |
| 39 C | " " ..... 10 | 23.1 ± 0.331 | 9.58 | 17 |
| 40 C | " " ..... 1 | 26.7 ± 0.437 | 10.00 | 17 |
| 41 C | Remainder of kernel..... 20 | 21.6 ± 0.154 | 4.27 | 19 |
| 42 C | " " " ..... 10 | 26.5 ± 0.443 | 9.28 | 14 |
| 43 C | " " " ..... 1 | 38.6 ± 1.37 | 14.94 | 8 |

* Coefficient of variability.

is drawn from the great drop in growth rate when Rations 30 C and 33 C are compared. The small number of pupæ on Ration 34 C does not permit its comparison with Ration 31 C. This conclusive evidence that the embryo and its adjacent endosperm are richest in this vitamin duplicates the findings of Bell and Mendel in the work just mentioned.

For some undetermined reason corn does not supplement this basal ration so well as wheat (Table X). 10 per cent of whole corn is equal in growth-promoting power to 20 per cent but the rate is still 25 per cent below that with wheat. Results on the 1 per cent levels show a superiority for the germ in supplementary value but

the remainder of the kernel is surprisingly good considering the results reported by Croll and Mendel. They state that practically all of the vitamin B in corn is in the embryo. It is possible that this contradiction involves the plurality of the vitamin B factor and in any case the growth-promoting difference demonstrated between wheat and corn is related to this question. Further research is needed to clear up this point.

## DISCUSSION.

It has been suggested that investigations of the phylogenetic development of nutritional requirements might yield valuable implications in the understanding of needs of higher animals. From this work it seems that *Tribolium confusum* has no absolute requirement for fat-soluble substances for growth and reproduction of one generation at least. (The adults emerging in different types of rations including one of purified constituents with the alcoholic extract of wheat germ and no fat were held for a time and the appearance of larvæ was later noted in all.) Sherman and Storms (26) concluded from results with rats that there was no good evidence of synthesis of vitamin A in the animal body at any age. It should be possible to rear these larvæ on a fat-free or vitamin A-free diet in sufficient numbers to determine their content of this substance. Culturing intestinal contents on several types of media failed to reveal any consistently occurring organism that might be a symbiotic source of nutritional constituents. When the larvæ or various types of rations were exposed to daily ultraviolet irradiation no effect was detectable. Evidently this species either possesses remarkable synthetic ability or the physiologic functions whose regulation in mammals is concerned with these vitamins have a different mechanism.

The vitamin B type of accessory substance was not only a necessary constituent of the ration of *Tribolium* but was present in the larval bodies in sufficient amount to produce a fair rate of growth in rats when 0.5 gm. was fed daily. Absence of vitamin C requirement is, of course, not peculiar to this species. In general such wide differences in vitamin requirements between animals of different phyla lend further weight to the view that these substances are so widely different in composition and function that their present classification under one name will in the future be discarded.

This work has especially indicated the utility of *Tribolium* in vitamin experiments but its ease of manipulation on purified diets and small food requirements point to possibilities in such other biological analyses as those of protein values.

## CONCLUSIONS.

1. It is possible to determine the vitamin requirements of an insect, *Tribolium confusum*, a common pest in such human food materials as the cereals, by using purified or otherwise modified rations with essentially the procedure followed with the ordinary laboratory animals.

2. This insect can be rapidly reared on a purified ration of protein, salts, dextrin, and a small fraction of yeast, wheat germ, and their alcoholic extracts, though the latter are not so completely supplementary as the former. The addition of a fat with or without vitamin A accelerates the growth rate.

3. The distribution of the necessary growth-promoting substance is similar to that of vitamin B as defined by mammalian growth behavior and it is at least in part identical with the purified products of the Osborne and Wakeman, and Levene and van der Hoeven purification processes. Some evidence that a plural substance is concerned is pointed out.

4. *Tribolium* is sensitive qualitatively to as low as 0.5 per cent of a source of this growth-promoting substance or substances and apparently makes a quantitative response to its additions over a certain range.

5. Growth on fractions of wheat, corn, oats, barley, and rice indicates no absolutely limiting factor in any fraction tried.

6. When wheat and its fractions are added to a purified basal ration lacking vitamin B the embryo and adjacent portion of the kernel are shown to be the most abundantly supplied with this factor. A similar study with corn demonstrated the presence of the necessary vitamin in the endosperm.

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